

FARM PTO-1390  TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE  ATTORNEY'S DOCKET NUMBER  4121-126
		U S APPLICATION NO (If known, see 37 CFR 1.5)  <b>09/889182</b>
INTERNATIONAL APPLICATION NO.  PCT/DE00/00079	INTERNATIONAL FILING DATE  11 January 2000	PRIORITY DATE CLAIMED  11 January 1999
TITLE OF INVENTION  <b>SELECTION OF MONOCLOINAL ANTIBODIES</b>		
APPLICANT(S) FOR DO/EO/US  Frank Breitling, Annemarie Poustka and Gerard Moldenhauer		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</li> <li>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))             <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</li> <li>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input type="checkbox"/> have been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).*(<b>Unsigned</b>)</li> <li>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li> </ol>		
<b>Items 11. to 16. below concern other document(s) or information included:</b>		
<ol style="list-style-type: none"> <li>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98</li> <li>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>13. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li>14. <input type="checkbox"/> A substitute specification.</li> <li>15. <input checked="" type="checkbox"/> A small entity statement.</li> <li>16. <input type="checkbox"/> Other items or information: EPO Search Report in German</li> </ol>		

NOTE: This application is being filed with an unsigned Oath or Declaration under the provisions of 37 CFR § 1.53 in order that applicants may secure a filing date of July 10, 2001. Upon receipt of a "Notice to File Missing Parts - Filing Date Granted," a executed Declaration and Power of Attorney will be forwarded. The undersigned agent affirmatively states that she has been duly authorized and appointed to file this application on behalf of the applicants and applicants' assignees, and that the Declaration and Power of Attorney to be filed hereafter will confirm the undersigned agent's authorization and appointment. Applicants are considered a small entity and assignee Deutsches Krebsforschungszentrum is also considered a small entity within the meaning of 37 CFR § 1.9.

U71889182

17. <input checked="" type="checkbox"/> The following fees are submitted: <b>Basic National Fee</b> (37 CFR 1.492(a)(1)-(5)): <span style="float: right;">\$860.00</span> Search Report has been prepared by the EPO or JPO .....  International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... \$0.00 No International preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ..... \$0.00  Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$1000.00  International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ..... \$0.00				CALCULATIONS	PTO USE ONLY
				<b>JC18 Rec'd PCT/PTO 10 JUL 2001</b>	
<b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>\$ 860.00</b>	
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				<b>\$</b>	
Claims	Number Filed	Number Extra	Rate		
Total Claims	20-20 =	0	X \$18.00	<b>\$</b>	
Independent Claims	2-3 =	0	X \$80.00	<b>\$</b>	
Multiple dependent claim(s) (if applicable)			+ \$270.00	<b>\$</b>	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				<b>860.00</b>	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				<b>\$ 430.00</b>	
<b>SUBTOTAL =</b>				<b>\$ 430.00</b>	
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				<b>\$</b>	
<b>TOTAL NATIONAL FEE =</b>				<b>\$ 430.00</b>	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				<b>\$</b>	
<b>TOTAL FEE ENCLOSED =</b>				<b>\$ 430.00</b>	
				<b>Amount to be:</b>	<b>\$</b>
				<b>refunded</b>	
				<b>Charged</b>	<b>\$</b>
a. <input checked="" type="checkbox"/> A check in the amount of <u>\$430.00</u> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>08-3284</u> . A duplicate copy of this sheet is enclosed.					
<b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not yet been met, a petition to revive (37 CFR 1.127(a) or (b)) must be filed and granted to restore the application to pending status.</b>					
SEND ALL CORRESPONDENCE TO:  <b>Steven J. Hultquist</b> <b>Intellectual Property/Technology Law</b> <b>P. O. Box 14329</b> <b>Research Triangle Park, NC 27709</b>					
 <b>MARIANNE FUERER</b> <b>Registration No. 39,983</b>					

09/889182

CALCULATIONS PTO USE ONLY

JC18 Rec'd PCT/PTO 10 JUL 2001

17.  The following fees are submitted:

**Basic National Fee** (37 CFR 1.492(a)(1)-(5)):

Search Report has been prepared by the EPO or JPO ..... \$860.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... \$0.00  
No International preliminary examination fee paid to USPTO (37 CFR 1.482)  
but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ..... \$0.00

Neither international preliminary examination fee (37 CFR 1.482) nor  
international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$1000.00

International preliminary examination fee paid to USPTO (37 CFR 1.482)  
and all claims satisfied provisions of PCT Article 33(2)-(4). ..... \$0.00

**ENTER APPROPRIATE BASIC FEE AMOUNT =**

\$ 860.00

Surcharge of **\$130.00** for furnishing the oath or declaration later than  20  30 months from the earliest claimed priority date (37 CFR 1.492(e)).

\$

Claims	Number Filed	Number Extra	Rate	
Total Claims	20-20 =	0	X \$18.00	\$
Independent Claims	2-3 =	0	X \$80.00	\$
Multiple dependent claim(s) (if applicable)			+ \$270.00	\$

**TOTAL OF ABOVE CALCULATIONS =** 860.00

Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).

**SUBTOTAL =** \$ 430.00

Processing fee of **\$130.00** for furnishing the English translation later than  20  30 Months from the earliest claimed priority date (37 CFR 1.492(f)).

\$

+

**TOTAL NATIONAL FEE =** \$ 430.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property

\$

+

**TOTAL FEE ENCLOSED =** \$ 430.00

Amount to be:  
refunded

Charged

- a.  A check in the amount of \$430.00 to cover the above fees is enclosed.
- b.  Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_ to cover the above fees.  
A duplicate copy of this sheet is enclosed.
- c.  The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-3284. A duplicate copy of this sheet is enclosed.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not yet been met, a petition to revive (37 CFR 1.127(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

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*Marianne Fuerer*  
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JO14 Rec'd PCT/PTO 03 JAN 2002

PCT

4121-126

PATENT APPLICATION

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re Application of: BREITLING, et al. 

Application No.: 09/889,182 23448  
PATENT & TRADEMARK OFFICE

International Application No.: PCT/DE00/00079

Priority Date Claimed: 11 January 2000 and 11 January 1999 (German Appl. No. 199 00 635.0)

Title: SELECTION OF MONOCLOINAL ANTIBODIES

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FIRST CLASS MAIL CERTIFICATE

I hereby certify that I am mailing the attached documents to the Commissioner for Patents on the date specified, in an envelope addressed to the Commissioner for Patents, Washington, DC 20231, and First Class Mailed under the provisions of 37 CFR 1.8.



Lee Ann Brown

November 14, 2001

Date of Mailing

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SUPPLEMENTAL PRELIMINARY AMENDMENT

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Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified national phase patent application, please amend the application, as follows:

In the Specification

Please insert on page 1 between the title of the application and the first paragraph the following new paragraph:



#### CROSS-REFERENCE TO RELATED APPLICATIONS

This application is filed under the provisions of 35 U. S.C. §371 and claims the priority of International Patent Application No. PCT/DE00/00079 filed January 11, 2000, and which in turn claims priority of German Patent Application No. 199 00 635.0 filed January 11, 1999.

#### REMARKS

This claim to priority is being filed before the above-identified application meets all requirements under 35 U.S.C. §371(b).

Respectfully submitted,

  
Marianne Fuierer  
Registration No. 39,983  
Attorney for Applicants

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09/889182  
JC18 Rec'd PCT/PTO 10 JUL 2001

4121-126  
PATENT APPLICATION

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re Application of: BREITLING, et al.  
Application No.: New U.S. National Stage Application of  
PCT International Application No.  
PCT/DE00/00079  
International Filing Date: 11 January 2000  
Priority Date Claimed: 11 January 1999 (German Appl. No. 199 00  
635.0)  
U.S. National Phase Filing Date: Date of mailing identified below  
Title: **SELECTION OF MONOCLOINAL  
ANTIBODIES**

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EXPRESS MAIL CERTIFICATE

I hereby certify that I am mailing the attached documents to the  
Commissioner for Patents on the date specified, in an envelope  
addressed to the Commissioner for Patents, Box Patent Application,  
Washington, DC 20231, and Express Mailed under the provisions of  
37 CFR 1.10

Blake Crouch

Name of Person Mailing This Document



Signature

July 10, 2001

Date

EL666414295US

Express Mail Label Number

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**PRELIMINARY AMENDMENT**

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Commissioner for Patents  
BOX PATENT APPLICATION  
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified new national phase patent application, please amend the application, as follows:

**In the Specification**

On the bottom of page 4 and top of page 5, please replace the paragraph with the following paragraph:

Preferred antibody binding proteins are shown in figures 1 to 3. The antibody binding protein of figure 1 comprises the signal peptide of a mouse MHC class I k(k) molecule, four antibody binding domains of the L protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 3 from nucleotide 682-1782) and amino acid sequences (SEQ ID NO: 4) of the antibody binding protein are given between nucleotide numbers 682-1782. The antibody binding protein of figure 2 comprises the signal peptide of a mouse Ig kappa chain, two antibody binding sites of the G protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 1 from nucleotide 737-1420) and amino acid sequences (SEQ ID NO: 2) of the antibody binding protein are indicated between nucleotide numbers 737-1420. The antibody binding protein of figure 3 comprises the signal peptide of the mouse MHC class I k(k) molecule, two antibodies binding sites of the G protein and the transmembrane domain of PDGFR. The DNA (SEQ ID NO: 5 from nucleotide 682-1431) and amino acid sequences (SEQ ID NO: 6) of the antibody binding protein are given between nucleotide numbers 682-1431. The antibody binding sites of all three antibody binding proteins have, on a DNA level, codons which are optimized for expression in mammalian cells.

**In the Claims**

Please amend claims 1-20 to read as follows:

1. A method of selecting monoclonal antibodies, comprising the fusion of B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, the antibodies being presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and the binding of the antibodies to

antigens, wherein the antibody binding proteins are inserted in the hybridoma cells via the myeloma cells or in the hybridoma cells via the expression vectors coding therefor.

2. The method according to claim 1, wherein the antibody binding protein comprises a signal peptide, an antibody binding site independent of the antibody specificity and a membrane anchor.
3. The method according to claim 2, wherein the antibody binding protein comprises an Fc binding protein or portions thereof.
4. The method according to claim 2, wherein the antibody binding protein comprises a combination of Fc binding proteins or portions thereof.
5. The method according to claim 4, wherein the Fc binding protein is selected from the group consisting of CD16, CD32 and CD64.
6. The method according to claim 2, wherein the antibody binding protein comprises an antibody binding domain of proteins selected from the group consisting of A, G, L and LG.
7. The method according to claim 2, wherein the antibody binding protein comprises a combination of a signal peptide selected from the group consisting of a signal peptide of a mouse Ig kappa chain, and a signal peptide of a mouse MHC-class I k(k) molecule; an antibody binding site of a protein selected from the group consisting of protein A, G, L, and LG; and a transmembrane domain selected from the group consisting of PDGFR and CD52.
8. The method according to claim 7, wherein the antibody binding protein is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 6.

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9. The method according to claim 1, wherein the hybridoma cells (over)express Rag1 and/or Rag2.
10. The method according to claim 1, wherein the antigens originate from an antigen library.
11. The method according to claim 1, wherein the antigens are bound to a carrier.
12. The method according to claim 11, wherein the carrier comprises magnetobeads.
13. The method according to claim 7, wherein the antigens comprise a fluorescence or biotin labeling.
14. The method according to claim 13, wherein the fluorescence labeling comprises FITC, TRITC, Cy3, Cy5, Cy5.5, Cy7 and phycoerythrin.
15. An antibody binding protein, wherein the antibody binding protein comprises a combination of the signal peptide selected from the group consisting of a mouse Ig cappa chain and a mouse MHC-class I k(k) molecule, an antibody binding site of proteins selected from the group consisting of A, G, L and LG; and a transmembrane domain selected from the group consisting of PDGFR and CD52.
16. The antibody binding protein according to claim 15, wherein the antibody binding protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID.NO 4 and SEQ ID NO:6 or an amino acid sequence differing therefrom by one or more amino acids.
17. DNA coding for the antibody binding protein according to claim 16, comprising:  
(a) the DNA of an antibody binding protein selected from the group consisting of SEQ ID NO: 1 from nucleotide 737-1420, SEQ ID NO: 3 from nucleotide 682-1782, and SEQ ID NO: 5 from nucleotide 682-1431

- [of figure 1, 2 or 3], a DNA differing therefrom by one or more base pairs, or
- (b) a DNA related to the DNA of (a) via the degenerated code.
18. An expression vector, coding for the DNA according to claim 17.
19. Cells containing the expression vector according to claim 18.
20. An antibody directed against the antibody binding protein according to claim 16.

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**REMARKS**

A marked-up version of amended paragraph in the specification and amended claims 1-20 are included herewith in Appendix A.

It is requested that the examination and prosecution of this application proceed on the basis of the English translation of the PCT International application included herewith and these amended claims 1-20.

Respectfully submitted,



Marianne Fuerer  
Registration No. 39,983  
Attorney for Applicants

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Attorney File: 4121-126

## APPENDIX A

### In the Specification

On the bottom of page 4 and top of page 5, replace the paragraph with the following paragraph:

Preferred antibody binding proteins are shown in figures 1 to 3. The antibody binding protein of figure 1 comprises the signal peptide of a mouse MHC class I k(k) molecule, four antibody binding domains of the L protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 3 from nucleotide 682-1782) and amino acid sequences (SEQ ID. NO: 4) of the antibody binding protein are given between nucleotide numbers 682-1782. The antibody binding protein of figure 2 comprises the signal peptide of a mouse Ig kappa chain, two antibody binding sites of the G protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 1 from nucleotide 737-1420) and amino acid sequences (SEQ ID NO: 2) of the antibody binding protein are indicated between nucleotide numbers 737-1420. The antibody binding protein of figure 3 comprises the signal peptide of the mouse MHC class I k(k) molecule, two antibodies binding sites of the G protein and the transmembrane domain of PDGFR. The DNA (SEQ ID NO: 5 from nucleotide 682-1431)and amino acid sequences (SEQ ID NO: 6) of the antibody binding protein are given between nucleotide numbers 682-1431. The antibody binding sites of all three antibody binding proteins have, on a DNA level, codons which are optimized for expression in mammalian cells.

### In the Claims

5. The method according to claim [3 or] 4, wherein the Fc binding protein is selected from the group consisting of CD16, CD32 [or] and CD64.
  
6. The method according to claim 2 [any of claims 2 to 5], wherein the antibody binding protein comprises an antibody binding domain of proteins selected from the group consisting of A, G, L and [or] LG.

7. The method according to claim 2, wherein the antibody binding protein comprises a combination of a [the] signal peptide selected from the group consisting of a signal peptide of a mouse Ig cappa chain, and a signal peptide of a mouse MHC-class I k(k) molecule; an antibody binding site of a protein[s] selected from the group consisting of protein A, G, L, and [or] LG; and [the] a transmembrane domain selected from the group consisting of PDGFR [or] and CD52.
8. The method according to claim 7, wherein the antibody binding protein is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 6. [that of figure 1, figure 2 or figure 3.]
9. The method according to claim 1 [any of claims 1 to 8], wherein the hybridoma cells (over)express Rag1 and/or Rag2.
10. The method according to claim 1 [any of claims 1 to 9], wherein the antigens originate from an antigen library.
11. The method according to claim 1 [any of claims 1 to 10], wherein the antigens are bound to a carrier.
13. The method according to claim 7, [any of claims 1 to 10], wherein the antigens comprise a fluorescence or biotin labeling.
15. An antibody binding protein, wherein the antibody binding protein comprises a combination of the signal peptide selected from the group consisting of a mouse Ig cappa chain [or] and a mouse MHC-class I k(k) molecule, an antibody binding site of proteins selected from the group consisting of A, G, L [or] and LG and a [the] transmembrane domain selected from the group consisting of PDGFR [or] and CD52.

16. The antibody binding protein according to claim 15, wherein the antibody binding protein comprises an [the] amino acid sequence selected from the group consisting of [figure 1, figure 2 or figure 3] SEQ ID NO: 2, SEQ ID.NO 4 and SEQ ID NO:6 or an amino acid sequence differing therefrom by one or more amino acids.
17. DNA coding for the antibody binding protein according to claim 16, comprising:
  - (a) the DNA of an antibody binding protein selected from the group consisting of SEQ ID NO: 1 from nucleotide 737-1420, SEQ ID NO: 3 from nucleotide 682-1782, and SEQ ID NO: 5 from nucleotide 682-1431 [of figure 1, 2 or 3], a DNA differing therefrom by one or more base pairs, or
  - (b) a DNA related to the DNA of (a) via the degenerated code.

DETAILED ACTION

18/PR1S

09/889182  
JC18 Rec'd PCT/PTO 10 JUL 2001

### **Selection of Monoclonal Antibodies**

The present invention relates to a method of selecting monoclonal antibodies and to means which can be used therefor.

The production of monoclonal antibodies is based on a method developed by Kohler and Milstein. According to this method B lymphocytes are fused with myeloma cells so as to obtain antibody-producing hybridoma cells. Such a method comprises major drawbacks. In particular, it is time-consuming and expensive to select antibodies, since this calls for separate culturing of hybridoma cells. Due to the latter only a limited number of hybridoma cells is detected and thus not all of the antibodies can be selected, this being a drawback in particular when antibodies with maximum affinity for an antigen shall be selected.

It is thus the object of the present invention to provide a product by which monoclonal antibodies can be produced, the above drawbacks being avoided.

According to the invention this is achieved by the subject matters defined in the claims.

The present invention is based on Applicant's insights that monoclonal antibodies on the cell surface of hybridoma cells can be presented by means of an antibody binding protein. He realized that monoclonal antibodies can be selected by this without hybridoma cells having to be cultured separately. He also realized that monoclonal antibodies can be selected

with respect to a determined and many (un)determined antigens of an antigen library. Furthermore, he found that monoclonal antibodies can also be selected with respect to their affinity intensity for certain antigens.

According to the invention Applicant's insights are used to provide a method of selecting monoclonal antibodies. Such a method comprises fusing B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, the antibodies being presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and binding of the antibodies to antigens.

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The expression "B lymphocytes" comprises B lymphocytes of any kind and origin. They may also concern precursors of B lymphocytes. The B lymphocytes may originate from animals, such as mice, rats, rabbits, etc., or humans. The B lymphocytes may also originate from a healthy or diseased organism. It is favorable for them to originate from an immunized organism. It is particularly favorable for the B lymphocytes to code for human antibodies or portions thereof. If B lymphocytes from animals are concerned, this can be achieved when the animals are transgenic for the human antibodies or portions thereof. Such animals can be produced by common methods, it being an obvious thing to introduce the genes for the human antibodies or the portions thereof into embryonal stem cells from which the animals are then generated. B lymphocytes and their precursors may be provided by common methods.

The expression "myeloma cells" comprises myeloma cells of any kind and origin. They may also concern precursors of myeloma cells. Furthermore, the myeloma cells may originate from animals, such as mice, rats, rabbits, etc., or humans.

Preferred myeloma cells are descendants from the mouse strains P3K, P3-X63.Ag8, X63.Ag8.653, NSO/1, Sp2/O-Ag14 and FO, the rat strains Y3-Ag1.2.3, YB2/0 and IR9834, and the human strains U266, SK007 and Karpas 707. Myeloma cells and their precursors can be provided by common methods.

The expression "antibody-producing hybridoma cells" comprises cells which form by fusion of B lymphocytes and myeloma cells and produce antibodies. Corresponding reference is made to the statements on B lymphocytes and myeloma cells. Hybridoma cells may include animal and/or human nucleic acids and/or proteins. Hybridoma cells can be cultured by common methods. It may also be favorable for the hybridoma cells to (over)express recombinases, e.g. Rag1 or Rag2, and/or mutases. This can be achieved by transfection of the hybridoma cells with corresponding expression vectors. The person skilled in the art knows such expression vectors.

The term "fusion of B lymphocytes with myeloma cells" concerns any method by means of which these cells may be fused. A method is favorable in which the cells are fused via polyethylene glycol. Reference is made to the examples.

The term "binding of the antibodies to antigens" concerns any method by which the antibodies expressed on the cell surface of the hybridoma cells can bind to antigens. The antigens can be bound to carriers, e.g. magnetobeads. They can also be labeled, e.g. fluorescence-labeled. For example FITC, TRITC, Cy3, Cy5, Cy5.5, Cy7 and phycoerythrin offer themselves as fluorescence markers. The antigens may also be coupled to biotin. Bound antigens may be detected by common methods, e.g. FACS analysis whereby the corresponding

DECODED  
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antibodies are also detected. Reference is made to the examples.

The expression "antibody binding protein" comprises any protein which may bind an antibody and present it on the cell surface of hybridoma cells. In particular, the protein may have a signal peptide, an antibody-binding site independent of the specificity of the antibody and a membrane anchor. Examples of such a protein are natural Fc binding proteins, such as CD16, CD32 and CD64. The protein may comprise a combination of a signal peptide, an antibody binding site and a membrane anchor, which does not occur in nature. Such a combination may comprise portions of natural Fc binding proteins. Furthermore, as a signal peptide it may have one of a mouse Ig kappa chain or a mouse MHC-class I k(k) molecule, as a membrane anchor it may include a transmembrane domain of PDGRF or CD52 and as an antibody binding site it may comprise an antigen binding domain of a bacterial protein, such as protein A, protein G, protein L or protein LG. It may be favorable for the combination to comprise several signal peptides, antibody binding sites and/or membrane anchors. It may be particularly favorable for the antibody binding protein, in particular the antibody binding domain of the bacterial proteins, to have codons which are optimized for expression in mammalian cells. A person skilled in the art knows which codons are concerned here.

Preferred antibody binding proteins are shown in figures 1 to 3. The antibody binding protein of figure 1 comprises the signal peptide of a mouse MHC class I k(k) molecule, four antibody binding domains of the L protein and the transmembrane domain of CD52. The DNA and amino acid sequences of the antibody binding protein are given between

DEPARTMENT OF TRADE AND INDUSTRY

nucleotide numbers 682-1782. The antibody binding protein of figure 2 comprises the signal peptide of a mouse Ig kappa chain, two antibody binding sites of the G protein and the transmembrane domain of CD52. The DNA and amino acid sequences of the antibody binding protein are indicated between nucleotide numbers 737-1420. The antibody binding protein of figure 3 comprises the signal peptide of the mouse MHC class I k(k) molecule, two antibodies binding sites of the G protein and the transmembrane domain of PDGFR. The DNA and amino acid sequences of the antibody binding protein are given between nucleotide numbers 682-1431. The antibody binding sites of all three antibody binding proteins have, on a DNA level, codons which are optimized for expression in mammalian cells.

An antibody binding protein of figures 1, 2 or 3 may have an amino acid sequence which differs from the amino acid sequence in figure 1, 2 or 3 by one or more amino acids. The differences may lie in additions, deletions, substitutions and/or inversions of individual amino acids. However, the DNA of this antibody binding protein hybridizes with the DNA indicated in figure 1, 2 or 3. The term "hybridizing" refers to hybridization under common conditions, in particular at 20°C below the melting point of the DNA. Furthermore, the antibody binding protein having the modified amino acid sequence comprises whole or partial functions which can be compared with those of the antibody binding protein of figure 1, 2 or 3.

Another subject matter of the present invention relates to a nucleic acid which codes for an above antibody binding protein. The nucleic acid may be an RNA or a DNA. Preferred is a DNA which comprises the following:

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- (a) the DNA of an antibody binding protein of figure 1, 2 or 3, a DNA differing therefrom by one or more base pairs, or
- (b) a DNA related to the DNA from (a) by the degenerated genetic code.

The term "a DNA differing by one or more base pairs" comprises any DNA coding for an antibody binding protein of figure 1, 2 or 3, which hybridizes with the DNA of figure 1, 2 or 3. The differences may lie in additions, deletions, substitutions and/or inversions of individual base pairs. As to the term "hybridizing" reference is made to the above explanations.

A DNA according to the invention may be present as such or in combination with any other DNA. In particular, a DNA according to the invention, which codes for an antibody binding protein, may be present in an expression vector. The person skilled in the art is familiar with examples thereof. In the case of an expression vector for *E. coli* these are e.g. pGEMEX, pUC derivatives, pGEX-2T, pET3b and pQE-8. For the expression in yeast, e.g. pY100 and Ycpad1 have to be mentioned while e.g. pKCR, pEFBOS, pCDM8 and pCEV4 have to be indicated for the expression in animal cells. The baculovirus expression vector pAcSGHisNT-A is particularly suitable for the expression in insect cells.

The person skilled in the art knows how to insert the DNA according to the invention in an expression vector. He also knows that this DNA can be inserted in combination with a DNA coding for another protein or peptide, so that the DNA according to the invention can be expressed in the form of a fusion protein.

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Preferred expression vectors which contain a DNA according to the invention are shown in figures 1 to 3. The expression vectors pSEX11L4, pSEX11G2\* and pSEX15G2 are concerned. They were deposited with the DSMZ (*Deutsche Sammlung für Mikroorganismen und Zellkulturen* [German-type collection of microorganisms and cell cultures]) on December 14, 1998. In particular, pSEX11L4 was deposited under DSM 12580, pSex11G2\* was deposited under DSM 12581 and pSEX15G2 was deposited under DSM 12582.

The person skilled in the art is familiar with suitable cells to express a cDNA according to the invention, which is present in an expression vector. Examples of such cells comprise the *E. coli* strains XL-1 Blue, Top 10 F, HB101, DH5alpha, x1776, JM101, JM 109, BL21 and SG 13009, the yeast strain *Saccharomyces cerevisiae* and *Pichia pastoris*, the animal cells L, NIH 3T3, FM3A, CHO, COS, Vero, HeLa, myeloma and hybridoma cells as well as the insect cells sf9.

The person skilled in the art also knows conditions of culturing transformed or transfected cells. He is also familiar with methods of isolating and purifying the protein or fusion protein expressed by the cDNA according to the invention.

Another subject matter of the present invention relates to an antibody directed against an above protein or fusion protein. Such an antibody may be prepared by common methods. It may be polyclonal or monoclonal. For its preparation it is favorable to immunize animals - in particular rabbits or chickens for a polyclonal antibody and mice for a monoclonal antibody - with an above (fusion) protein or with fragments thereof. Further "boosters" of the animals can be effected

with the same (fusion) protein or with fragments thereof. The polyclonal antibody may then be obtained from the animal serum or egg. For the preparation of the monoclonal antibody, animal spleen cells are fused with myeloma cells.

Another subject matter of the present invention is a kit. Such a kit comprises one or more of the following components:

- (a) a DNA according to the invention,
- (b) a cell expressing a DNA according to the invention,
- (c) an antibody binding protein according to the invention,
- (d) an antibody according to the invention, and
- (e) common auxiliary substances such as carriers, buffers, solvents, controls, markers, detection reagents for components (a) - (d).

One or more representatives of the individual components may be present. As to the individual terms reference is made to the above statements. They apply here analogously.

The present invention distinguishes itself in that antibodies produced by hybridoma cells are presented on the cell surface of the hybridoma cells. This is done via an antibody binding protein. Such a protein may be introduced into the hybridoma cells via the myeloma cells used for the production of the hybridoma cells. The antibody binding protein may also be introduced into the hybridoma cells via an expression vector coding for it.

By means of the present invention it is possible to select antibodies. This can be done without much expenditure, since hybridoma cells do not have to be cultured separately. Complex mixtures of hybridoma cells can rather be used

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directly for selecting antibodies. Antibodies can also be selected for their affinity intensity for certain antigens. The present invention is also suitable for selecting antibodies of hybridoma cell libraries, not only with respect to a determined antigen but also as regards many (un)determined antigens of antigen libraries.

Thus, the present invention provides products by which it is possible to avoid *inter alia* major problems as regards time and cost which have occurred in the selection of monoclonal antibodies thus far.

**Brief description of the drawings:**

Fig. 1 shows the expression vector pSEX11L4 according to the invention (figure 1A) which codes for an antibody binding protein (figure 1(B)). Reference is made to the above explanations.

Fig. 3 shows the expression vector pSEX11G2\* according to the invention (figure 2(A)), which codes for an antibody binding protein (figure 2(B)). Reference is made to the above explanations.

Figure 3 shows the expression vector pSEX15G2 according to the invention (figure 3(A)), which codes for an antibody binding protein (figure 3(B)). Reference is made to the above explanations.

The present invention is explained by the below examples.

**Example 1: Preparation of myeloma cells which express an antibody binding protein on their cell surface**

**(A) Transient expression**

Cells of the myeloma cell line X63-Ag8.653 are used. These cells ( $10^7$ ) are transfected with 20-40 µg of the expression vector SEX11G2\* according to the invention (cf. figure 2). Electroporation is carried out as transfection technique, which comprises two pulses of 2 ms at 500 V. The cells are incubated for 48 h at 37°C and 5-7.5 % Co<sub>2</sub> in RPMI medium which contains 10 % FCS. Thereafter, the cells are washed with cold DPBS + 0.1 % Na azide before they are incubated for 45 minutes at 0°C with DPBS + 0.1 % Na azide plus 25 µg/ml goat anti-calf antibody (FITC-labeled; GAB-FITC, Dianova company). Having been washed with DPBS + 0.1 % Na azide, the cells are incubated in DPBS + 0.1 % Na azide + 1 µg/ml propidium iodide and subjected to FACS analysis following excitation with blue light.

It shows that the transfected myeloma cells have a green fluorescence which is due to the transient expression of an antibody binding protein on the cell surface of the myeloma cells.

**(B) Stable expression**

The myeloma cells obtained under (A) are subjected to G418 selection for 14-24 days before they are incubated using GAB-FITC and subjected to FACS analysis as described under (A). Myeloma cells which have a strong green fluorescence are subject to further G418 selection rounds or runs.

The myeloma cell line X63-Ag8.653.3 is obtained which stably expresses an antibody binding protein on its cell surface.

**Example 2: Production of hybridoma cells which express on their cell surface antibodies by means of an antibody binding protein**

**(A)**

10 Balb/c mice are immunized subcutaneously in each case with 100 µg killed *Helicobacter pylori* bacteria in complete Freund's adjuvant, which contains killed *Mycobacter tuberculosis* bacteria. After 4 or 7 weeks, an intraperitoneal booster injection with 100 µg killed *Helicobacter pylori/Mycobacter tuberculosis* bacteria is given. 100 µl blood serum are withdrawn from the mice before each immunization and after the last immunization, and the antigen-specific immune response of the mouse is tested in a Western blot. A degradation of bacterial whole protein of *Helicobacter pylori* and/or *Mycobacter tuberculosis* is used as antigen. The detection of bound mouse antibodies is made by an peroxidase-conjugated goat anti-mouse antibody (Dianova company). The spleen of mice having a marked antigen-specific immune response is removed and the lymphocytes are fused with cells of the myeloma cell line X63-Ag8.653.3 of Example 1 (B). The fusion is made by means of polyethylene glycol (cf. Goding, J.W., Cell Biology, Biochemistry and Immunology, 3<sup>rd</sup> edition (1996), Verlag Academic Press Limited, 24-28). Hybridoma cells are obtained. They are incubated in HAT medium at 37°C for 10 to 12 days. The hybridoma cell library 2A is obtained.

Hexapeptides with N-terminal biotin are synthesized. The peptides correspond to the 6C-terminal amino acids of 101 or 118 gene products of *Helicobacter pylory* or *Mycobacter*

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tuberculosis.  $10^3$  cells of the hybridoma cell library 2A are also washed with cold DPBS + 0.1 % Na azide and incubated for 45 minutes at 0°C with DPBS + 0.1 % Na azide + 10 µg/ml of the above biotin-labeled peptides. The cells are washed with cold DPBS + 0.1 % Na azide and incubated for 45 minutes at 0°C with 10 µg/ml streptavidine FITC. Having been washed with DPBS + 0.1 % Na azide, the cells are incubated in DPBS + 0.1 % Na azide + 1 µg/ml propidium iodide and subjected to FACS analysis after excitation with blue light.

It shows that the hybridoma cells have a green fluorescence. This fluorescence is due to the expression of antibodies on the cell surface of the hybridoma cells. Further studies show that the antibodies have an anti-*Helicobacter pylori* or *Mycobacter tuberculosis* activity.

**(B)**

Cells of the hybridoma cell line U98/6 which produce a mouse anti-urokinase antibody are used. These cells ( $10^7$ ) are transfected with 20-40 µg of the pSEX11G2\* expression vector according to the invention (cf. figure 2). Electroporation is carried out as a transfection technique, which comprises two pulses of 2 ms at 400 V. The cells are incubated for 48 h in incomplete AIM V-medium at 37°C and 5-7.5 % Co<sub>2</sub>. Thereafter, the cells are washed with cold DPBS + 0.1 % Na azide before they are incubated at 0°C for 45 minutes with DPBS + 0.1 % Na azide + 10 µg/ml urokinase biotin. Having been washed with DPBS + 0.1 % Na azide, the cells are incubated in DPBS + 0.1 % Na azide + 10 µg/ml streptavidine FITC and subjected to FACS analysis after excitation with blue light.

It shows that the transfected hybridoma cells have a green fluorescence. This fluorescence is due to the expression of

antibodies on the cell surface of the hybridoma cells. Further studies show that the antibodies have anti-urokinase activity.

The resulting hybridoma cells are subjected to G418 selection for 14 to 24 days before they are again incubated with urokinase-biotin and streptaavidine-FICS and subjected to FACS analysis as described above. Hybridoma cells which have a strong green fluorescence are subjected to further G418 selection rounds.

The hybridoma cell line U98/6.3.3 is obtained. It stably expresses antibodies on its cell surface.

**Example 3: Selection of monoclonal antibodies which are expressed on the cell surface of hybridoma cells by means of an antibody binding protein**

<sup>10<sup>3</sup></sup> cells of the hybridoma cell line U98/6.3.3 of Example 2 (B) are mixed with <sup>10<sup>7</sup></sup> cells of the hybridoma cell line DOB.L1.3. The latter hybridoma cell line produces an antibody recognizing the C terminus of the human HLA-DO-β chain. It is expressed on the cell surface by means of an antibody binding protein the same as that in the hybridoma cell line U98/6.3.3 of Example 2(B). The cell mixture is washed with cold DPBS + 0.1 % Na azide and incubated at 0°C for 45 minutes with DPBS + 0.1 % Na azide + 10 µg/ml urokinase biotin. Having been washed with DPBS + 0.1 % Na azide, the cell mixture is incubated in DPBS + 0.1 % Na azide + 10 µg/ml streptavidine FITC and supplied to a FACS sorter following excitation with blue light.

Hybridoma cells with green fluorescence are selected. In further studies, they show an anti-urokinase activity. The hybridoma cell lines U98/6.3.3 S1-S50 are obtained.

**Example 4: Production and purification of an antibody binding protein according to the invention**

**(A)**

The DNA of figure 1 between nucleotide numbers 682-1782 is provided with BAMHI linkers, subsequently cleaved using BamHI, and inserted in the pQE-8 expression vector cleaved by BamHI (Qiagen company). The expression plasmid pQE-8/antibody binding protein is obtained. Such a plasmid codes for a fusion protein comprising 6 histidine residues (N terminus partner) and the antibody binding protein of fig. 1 according to the invention (C terminus partner). pQE-8/antibody binding protein is used for transforming *E. coli* SG 13009 (cf. Gottesman, S. et al., J. Bacteriol. 148, (1981), 265-273). The bacteria are cultured in an LB broth with 100 µg/ml ampicillin and 25 µg/ml kanamycin and induced with 60 µM isopropyl-β-D-thiogalactopyranoside (IPTG) for 4 h. Lysis of the bacteria is achieved by the addition of 6 M guanidine hydrochloride. Thereafter, chromatography (Ni-NTA resin) is carried out with the lysate in the presence of 8 M urea in accordance with the instructions from the manufacturer of the chromatography material (Qiagen company). The bound fusion protein is eluted in a buffer having a pH of 3.5. After its neutralization, the fusion protein is subjected to 18 % SDS polyacrylamide gel electrophoresis and stained with coomassie blue (cf. Thomas, J.O., and Kornberg, R.D., J. Mol. Biol. 149 (1975), 709-733).

It shows that an antibody binding protein (fusion protein) according to the invention can be prepared in highly pure form.

**(B)**

$10^8$  cells of the myeloma cell line X63-Ag8.653.3 obtained in Example 1 (B) are washed with PBS, taken up in PBS + 1 % Tween 20 and incubated on ice. Particulate cell components are separated by centrifugation at 30,000 g, and the supernatant is placed on an IgG sepharose column (IgG sepharose 6 Fast Flow Lab Pack from Pharmacia company). Unbound components are removed by washing and the antibody binding protein according to the invention is eluted in acidic pH.

Following its neutralization, the antibody binding protein is subjected to 18 % SDS polyacrylamide gel electrophoresis and stained using coomassie blue (see above).

It showed that an antibody binding protein (fusion protein) according to the invention can be obtained in highly pure form.

**Example 5: Preparation and detection of an antibody according to the invention**

A fusion protein of Example 4 according to the invention is subjected to 18 % SDS polyacrylamide gel electrophoresis. After staining the gel with 4 M sodium acetate, an about 41 kD band was excised from the gel and incubated in phosphate-buffered common salt solution. Gel pieces are sedimented before the protein concentration of the supernatant is determined by SDS polyacrylamide gel electrophoresis which

is followed by coomassie blue staining. Animals are immunized with the gel-purified fusion protein as follows:

**Immunization protocol for polyclonal antibodies in rabbits**

35 µg of gel-purified fusion protein in 0.7 ml PBS and 0.7 ml of complete or incomplete Freund's adjuvant were used per immunization:

- Day 0: 1<sup>st</sup> immunization (complete Freund's adjuvant)  
Day 14: 2<sup>nd</sup> immunization (incomplete Freund's adjuvant; icFA)  
Day 28: 3<sup>rd</sup> immunization (icFA)  
Day 56: 4<sup>th</sup> immunization (icFA)  
Day 80: bleeding to death.

The rabbit serum is tested in an immunoblot. For this purpose, a fusion protein of Example 4 according to the invention is subjected to SDS polyacrylamide gel electrophoresis and transferred to a nitrocellulose filter (cf. Khyse-Andersen, J., J. Biochem. Biophys. Meth. 10 (1984), 203-209). The Western blot analysis was carried out as described in Bock, C.-T. et al., Virus Genes 8, (1994), 215-229. For this purpose, the nitrocellulose filter is incubated with a first antibody at 37°C for one hour. This antibody is the rabbit serum (1:10000 in PBS). After several wash steps using PBS, the nitrocellulose filter is incubated with a second antibody. This antibody is an alkaline phosphatase-coupled monoclonal goat anti-rabbit IgG antibody (Dianova company) (1:5000) in PBS. 30 minutes of incubation at 37°C are followed by several wash steps using PBS and subsequently by the alkaline phosphatase detection reaction with developer solution (36 µM 5'-bromo-4-chloro-3-indolylphosphate, 400 µM nitro blue tetrazolium, 100 mM

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Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM MgCl<sub>2</sub>) at room temperature until bands are visible.

It shows that polyclonal antibodies according to the invention can be prepared.

#### **Immunization protocol for polyclonal antibodies in chickens**

40 µg of gel-purified fusion protein in 0.8 ml PBS and 0.8 ml of complete or incomplete Freund's adjuvant were used per immunization.

Day 0: 1<sup>st</sup> immunization (complete Freund's adjuvant)

Day 28: 2<sup>nd</sup> immunization (incomplete Freund's adjuvant; icFA)

Day 50: 3<sup>rd</sup> immunization (icFA)

Antibodies are extracted from egg yolk and tested in a Western blot. Polyclonal antibodies according to the invention are detected.

#### **Immunization protocol for monoclonal antibodies in mice**

12 µg of gel-purified fusion protein in 0.25 ml PBS and 0.25 ml of complete or incomplete Freund's adjuvant are used per immunization. The fusion protein is dissolved in 0.5 ml (without adjuvant) in the 4<sup>th</sup> immunization.

Day 0: 1<sup>st</sup> immunization (complete Freund's adjuvant)

Day 28: 2<sup>nd</sup> immunization (incomplete Freund's adjuvant; icFA)

Day 56: 3<sup>rd</sup> immunization (icFA)

Day 84: 4<sup>th</sup> immunization (PBS)

Day 87: fusion.

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Supernatants of hybridomas are tested in a Western blot. Monoclonal antibodies according to the invention are detected.

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**Amended Claims**

1. A method of selecting monoclonal antibodies, comprising the fusion of B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, the antibodies being presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and the binding of the antibodies to antigens, wherein the antibody binding proteins are inserted in the hybridoma cells via the myeloma cells or in the hybridoma cells via the expression vectors coding therefor.
2. The method according to claim 1, wherein the antibody binding protein comprises a signal peptide, an antibody binding site independent of the antibody specificity and a membrane anchor.
3. The method according to claim 2, wherein the antibody binding protein comprises an Fc binding protein or portions thereof.
4. The method according to claim 2, wherein the antibody binding protein comprises a combination of Fc binding proteins or portions thereof.
5. The method according to claim 3 or 4, wherein the Fc binding protein is CD16, CD32 or CD64.
6. The method according to any of claims 2 to 5, wherein the antibody binding protein comprises an antibody binding domain of proteins A, G, L or LG.

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7. The method according to claim 2, wherein the antibody binding protein comprises a combination of the signal peptide of a mouse Ig kappa chain or a mouse MHC-class I k(k) molecule, an antibody binding site of proteins A, G, L or LG and the transmembrane domain of PDGFR or CD52.
8. The method according to claim 7, wherein the antibody binding protein is that of figure 1, figure 2 or figure 3.
9. The method according to any of claims 1 to 8, wherein the hybridoma cells (over)express Rag1 and/or Rag2.
10. The method according to any of claims 1 to 9, wherein the antigens originate from an antigen library.
11. The method according to any of claims 1 to 10, wherein the antigens are bound to a carrier.
12. The method according to claim 11, wherein the carrier comprises magnetobeads.
13. The method according to any of claims 1 to 10, wherein the antigens comprise a fluorescence or biotin labeling.
14. The method according to claim 13, wherein the fluorescence labeling comprises FITC, TRITC, Cy3, Cy5, Cy5.5, Cy7 and phycoerythrin.
15. An antibody binding protein, wherein the antibody binding protein comprises a combination of the signal peptide of a mouse Ig kappa chain or a mouse MHC-class

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I k(k) molecule, an antibody binding site of proteins A, G, L or LG and the transmembrane domain of PDGFR or CD52.

16. The antibody binding protein according to claim 15, wherein the antibody binding protein comprises the amino acid sequence of figure 1, figure 2 or figure 3 or an amino acid sequence differing therefrom by one or more amino acids.
17. DNA coding for the antibody binding protein according to claim 16, comprising:
  - (a) the DNA of an antibody binding protein of figure 1, 2 or 3, a DNA differing therefrom by one or more base pairs, or
  - (b) a DNA related to the DNA of (a) via the degenerated code.
18. An expression vector, coding for the DNA according to claim 17.
19. Cells containing the expression vector according to claim 18.
20. An antibody directed against the antibody binding protein according to claim 16.

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### **Abstract of the Disclosure**

The present invention relates to a method of selecting monoclonal antibodies, comprising the fusion of B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, wherein the antibodies are presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and to the binding of the antibodies to antigens. The invention also concerns means usable for this purpose.

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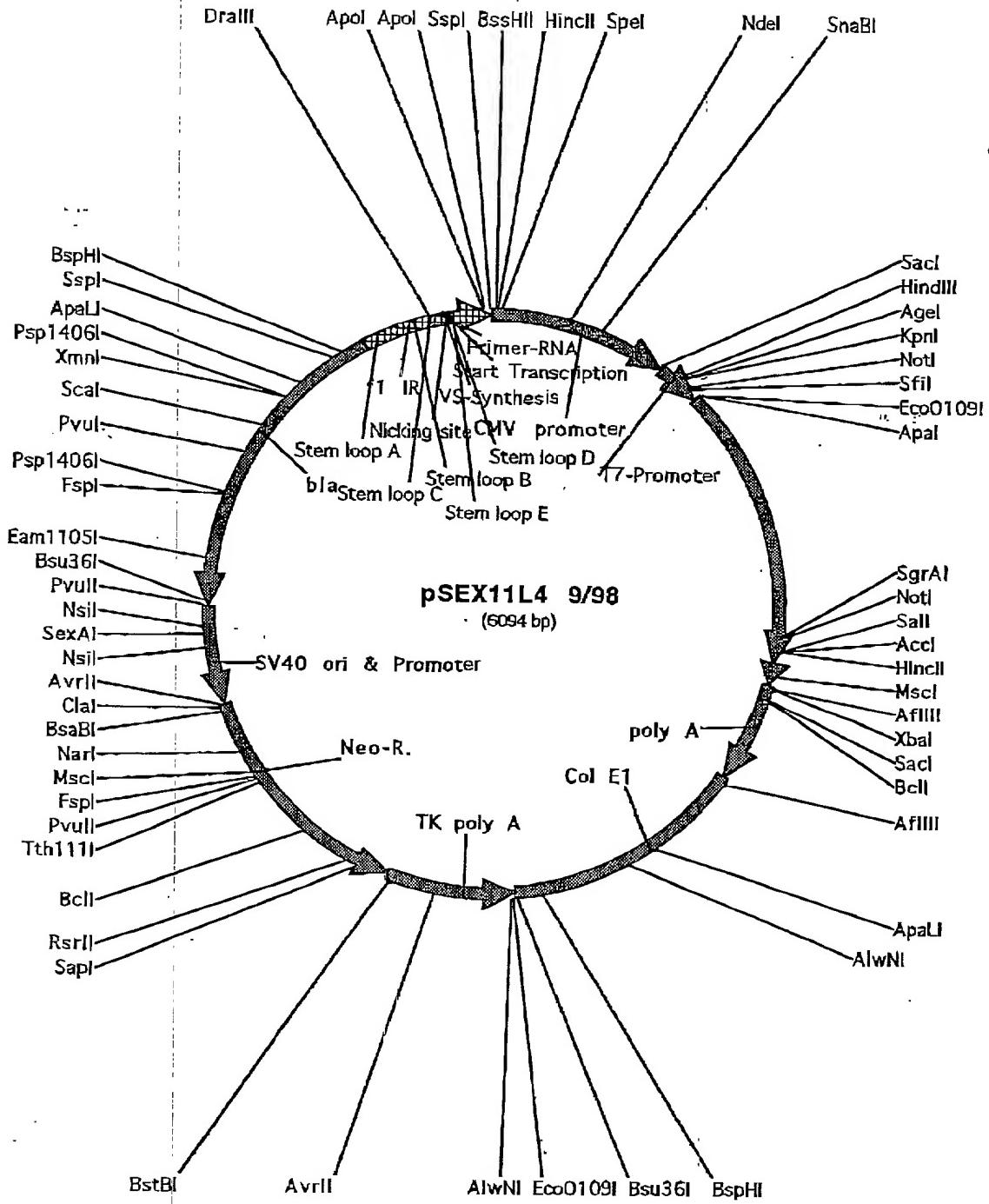


Fig. 1

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BbsHII HindIII SpeI

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1 GGGCGCGTTGACATTGATTATGACTAGTTATTAAATAGTAATCAATTACGGGTCTTAA

60 GTTCATAGCCCATAATGGAGTCCGCGTACATAACTTACGGTAAATGGCCGCCCTGG

119 CTGACCGCCCAACGACCCCCCCTTGCATGACGTCAATAATGACGTATGTTCCCATAGTAA

178 CGCCAATAGGGACTTCCATTGACGTCAATGGGTGACTATTTACGGTAAACTGCCAC

---

NdeI

237 TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGG

---

CMV promoter

296 TAAATGGCCCGCTGGCATTATGCCACTACATGACCTTATGGACTTTCTACTTGGC

---

SnaBI

355 AGTACATCTACGTATTAGTCATCGCTATTACCATGGTGTGCGGTTGGCAGTACATC

414 AATGGGCGTGGATAGCGGTTGACTCACGGGATTTCAAGTCTCCACCCATTGACGT

473 CAATGGGAGTTGTTGGACCAAAATCAACGGACTTCCAAAATGTCGAACAAC

---

532 CCGCCCCATTGACGCAAATGGCGGTAGGGTGTACGGTGGAGGTCTATATAAGCAGA SacI

---

T7-Pro

591 GCTCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAAACGACTCA

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Agel

HindIII KpnI

650 CTATAGGGAGACCCAAAGCTTGGTACCGGTGCGATGGCACCTGCATGCTCCTGCTG  
1> MetAlaProCysMetLeuLeuLeuLeu

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SfiI

NotI

ApaI

Eco0109I

709 TTGGCGGCCGCTGGCCCCACTCAGACCCCGCGGGGCCAAAAGGAGAACACCC  
10> LeuAlaAlaAlaLeuAlaProThrGIuThrArgAlaGlyAlaGluLysThrPr

768 CGAGGAGCCCAAGGAGGAGGTGACCATCAAGGCCAACCTGATCTACGGCGACGGCAAGA  
29> uGIuGIuPrOlysGIuGIuValThrIleTyrAlaAsnLeuIeTyrAlaAspGIuLysT

827 CCCGACCGCCGAGTTCAAGGGCACCTCGAGGGGCCACCCGGAGGGCTACCGCTAC  
49> rGIuThrAlaGluPhelysGIyThrPheGIuGIuAlaThrAlaGluAlaTyrArgTyr

886 CCCGACGCCCTGAAGAACGGAGACACGGCGAGTACACCGTGACGTGGCCGACAAGGGCTA  
69> AlaAspAlaLeuLysAspAsnGIyGIuTyThrValAspValAlaAspLysGIyTy

945 CACCCCTGAACATCAAGTTCGGCGCAAGGAGAACCCCCGAGGAGGCCAAGGAGGG  
88>rThrLeuAsnIleLysPheAlaGlyLysGIuLysThrPrAlaGluAluProLysGIuGIuV

1004 TGACCATCAAGGCCAACCTGATCTACGGCACGGCAAGACCCAGACCCGCGAGTTCAAG  
108>aIThrIleLysAlaAsnLeuIleTyrAlaAspGIuLysThrGIuThrAlaGluPhelys

1063 GGCACCTCGAGGGGCCACCGCGGAGGCCCTACCGCTACGCCGACGCCCTGAAGAAGGA  
128> GIyThrPheGIuGIuAlaThrAlaGluAlaTyrArgTyrAlaAspAlaLeuLysAs

1122 CAACGGCGAGTACACCGTGGACGTGGCCGACAAGGGCTACACCTGAACTAAGTTG  
147>pAsnGIyGIuTyThrValAspValAlaAspLysGIyTyrThrLeuAsnIleLysPheA

1181 CCGCGACAGGAGAACCCCCGAGGAGGCCAAGGAGGGATGACCTCAAGGCCAACCTG  
167> IaGlyLysGIuLysThrProGIuGIuProLysGIuGIuValThrIleLysAlaAsnLeu

1240 ATCTACGGCGACGGCAAGACCCAGACGGCGAGTTCAAGGGCACCTCGAGGGCAC  
187> IleTyrAlaAspGIuLysThrGIuThrAlaGluPhelysGIyThrPheGIuGIuAlaThr

1299 CGCGGAGGCCACCGCTACGCCGACGCCCTGAAGAAGGACAACGGCGAGTACACCGTGG  
206>rAlaGluAlaTyrArgTyrAlaAspAlaLeuLysAspAsnGIyGIuTyrThrValIA

1358 ACGTGGCCGACAAGGGCTACACCCCTGAACATCAAGTTCGGCGCAAGGAGAACCCCC  
226> spValAlaAspLysGIyTyrThrLeuAsnIleLysPheAlaGlyLysGIuLysThrPro

Fig. 1 (cont'd I)

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1417 GAGGAGCCAAGGGAGGTGACCATCAAGGCCAACCTGATCTACGCCACGCCAAGAC  
 246> GluGluProLysGluGluValThrIleLysAlaAsnLeuIleTyrAlaAspGlyLysTh  
 1476 CGAGACCGCCGAGTTCAAGGGCACCTTCGAGGAGGCCACCGCGAGGCCTACCGCTACG  
 265>rGlnThrAlaGluPheLysGlyThrPheGluGluValaThrAlaGluAlaTyrArgTyrA  
 1535 CCGACGCCCTGAAGAAGGACAACGGCGACTACCCGTGGACGTGGCCGACAAGGGCTAC  
 285> IaAspAlaLeuLysAspAsnGlyGluTyrThrValAspValAlaAspLysGlyTyr  
 SgrAl NotI  
 1594 ACCCTGAACATCAAGTTGCCGGCGGGCGCAGAACAAAAACTCATCTCAGAAGAGGA  
 305> ThrLeuAsnIleLysPheAlaGlyAlaAlaAlaGluGlnLysLeuIleSerGluGluAs

SallHincIIAcl

1653 TCTGAATGGGGCGTGCACGGACAAACGACACCAGCCAAACCAGCAGGCCCTCAGCAT  
 324>pLeuAsnGlyAlaValAspGlyGlyAlaAsnAspThrSerGlnThrSerSerProSerAlaS

MscI

1712 CCAGCAACATAAGCGGAGGCATTTCTTTCTTCGTGGCCATGCCATAATCCACCTC  
 344> SerAsnIleSerGlyGlyIlePheLeuPhePheValAlaAsnAlaAlaIleHisLeu

AfIII XbaI SacI  
 1771 TTCTGCTTCAGTTGAGGTGACAGTCAGAGCTATTCTATAGTGTACCTAAATGCTAG  
 364> PheCysPheSer \*\*\* ← →

BclI

1830 AGCTCGCTGATCAGCCTCGACTGTGCCCTCTAGTTGCCAGCCATCTGTTGTTGCCCT

← poly A

1889 CCCCCGTGCCCTCCTGACCCCTGGAAAGGTGCCACTCCACTGTCCCTTCTTAATAAAAT

1948 GAGGAAATTGCATCGCATTGTCAGTAGGTGTCATTCTATTCTGGGGGTGGGGTGGG

2007 GCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCCGTGG

2066 GCTCTATGGCTTCTGAGGGGAAAGAACCAAGTGGGGTAATAACGGTTATCCACAGAACTC  
AfIII

2125 AGGGATAAACCGCAGGAAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGAACCGTA

2184 AAAAGGCCGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGG

2243 AATCGACGCTCAAGTCAGAGGTGGCAAACCCGACAGGACTATAAGATAACAGGGTT

2302 TCCCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCCCTGCCGCTTACCGGATACC

2361 TGTCCGCCCTTCTCCCTCGGGAAAGGTGGCTTCTCATAGCTCACGCTGTAGGTAT.

ApaLI

2420 CTCAAGTTGGTGTAGGTGTTCGCTCAAGCTGGCTGTGTGCAAGAACCCCCGGTCA

Col E1

2479 GCGCGACCGCTGCCCTTATCCGTAACATATCGTCTTGAGTCCAACCCGGTAAGACACG

AlwNI

2538 ACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAACAGAGGCAGGATATGTAGGC

2597 GGTGCTACAGAGTTCTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGGACAGTATT

2656 TGGTATCTGGCTCTGCTGAAGCCAGTTACCTTGGAAAAAGAGTTGGTAGCTTGTAT

2715 CCGGCAAACAAACCAACCGCTGGTAGGGTGGTTTTTGTGCAAGCAGCAGATTACG

2774 CGCAGAAAAAAAGGATCTCAAGAAGATCTTGTATCTTCTACGGGTCTGACGGCTCA

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## BspHI

2833 GTGGAAACGAAAAACTCACGTTAAGGGATTTGGTCATGAGATTATCAAAAGGATCTTCA  
2892 CCTAGATCTTTAAATTAAAATGAAGTTTAAATCAATCTAAAGTATATGAGTAA

## EcoO109I

Bsu36I AlwNI  
2951 CCTGAGGCTATGGCAGGCCCTGCCGCCCGACGTTGGCTGCAGGCCCTGGCCCTCACC

3010 CGAACCTGGGGGTGGGGTGGGAAAAGGAAGAAACCGCGGGCGTATTGGCCCCAATGGG

3069 GTCCTCGTGGGTATCGACAGAGTGCAGCCCTGGACCGAACCCCGCGTTATGAACA

## TK poly A

3128 AACGACCCAACACCGTGCCTTTATTCTGCTTTTATTGCCGTATAGCGCGGGTTCC

## AvrII

3187 TCCCGGTATTGTCCTTCCGTGTTCAAGTAGCCCTCCCCCTAGGGTGGCGAAGAACT

3246 CCAGCATGAGATCCCCCGCTGGAGGATCATCCAGCCGGTCEGGAAAACGATTCCG  
3305 AAGCCCAACCTTCATAGAAGGCGGGCTGGAATCGAAATCTGTATGGCAGGTTGGG

## BstBI

3364 CGTCGCTTGGTCGGTCATTCGAACCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAG  
Z634 ••PhePheGl uAspLeuLeu

3423 GCGATAGAAGGCCATGGCCTGCGAATCGGGAGCGCGATACCGTAAGCACGGAGGAAGC  
256 ArgTyrPheAlaIleArgGlnSerAspProAlaAlaIleGlyTyrLeuValLeuPheAr

## SapI

3482 GGTCAAGCCCATTCGCCGCCAACAGCTCTCGAACATATCACGGGTAGCCAACGCTATGTCC  
2364 gAspAlaTrpGluGlyLeuGluGluValIleAspArgThrAlaLeuAlaIleAspG

## RsrII

3541 TGATAGCGGCCACACCCAGCCGCCACAGTCGATGAATCCAGAAAAGCGGCCATT  
2164 InTyrArgAspAlaValGlyLeuArgGlyCysAspIlePheGlySerPheArgGlyAsn

3600 TTCCACCATGATATTGCGCAAGCAGGATCGCCATGGTCACGACGGATCCTCGCCGT  
197 GluValMetIleAsnProLeuCysAlaAspGlyHisThrValValLeuAspGlyAsn

3659 CGGGCATGCTCGCCTTGAGCCCTGGCAACAGTTGGCTGGCGAGCCCCCTGATGCTCT  
177 pPheMetSerAlaLysLeuArgAlaPheLeuGluAlaProAlaLeuGlyGlnHisGluG

## BclI

3718 TGATCATCTGATCGACAAGACGGCTTCCATCCGAGTACGTCTCGCTCGATGCGATG  
1574 InAspAspGlnAspValLeuGlyAlaGlyMetArgThrArgAlaArgGlyIleArgGly

3777 TTTCGCTTGGTGGTCGAATGGGCAGGTAGGCCGATCAAGCGTATGCGCCGCATTG  
1384 LysAlaGlyHisAspPheProCysThrAlaProAspLeuThrGlyLeuArgArgMetAl

3836 CATCAGCCATGATGGATACTTCTGGCAGGAGCAAGGTGAGATGACAGGAGATCCCTGC  
1184 aAspAlaMetIleSerValLysGlyAlaProAlaLeuHisSerSerLeuLeuAspGlyG

## Tth111I

## PvuII

3895 CCCGGCACTTCGCCCAATAGCAGCCAGTCCCTCCGCTTCACTGACAACGTCGAGCAC  
984 IyProValGlyLeuLeuLeuTrpAspArgGlyAlaGlyThrValValAspLeuVal

## Neo-R.

FspI MscI  
3954 AGCTCGCAAGGAACGCCGTCGTGGCCAGCCACGATAGCCGCCGCTGCTCTGCA  
794 AlaAlaCysProValGlyThrThrAlaLeuTrpSerLeuArgAlaAlaGlyAspGlnLe

## NarI

4013 GTTCATTCAAGGGCACCGGACAGGTGGCTTGACAAAAAGAACGGGGGCCCTGGCT  
594 GlyAsnLeuAlaGlySerLeuAspThrLysValPheLeuValProArgGlyGlyAlaS

4072 GACAGCCGAAACACGCCGATCAGACGAGCCGATTGCTGTTGCCCAGTCATGCC  
394 SerLeuArgPheValAlaAlaAspSerCysGlyIleThrGlyGlyAlaTrpAspTyrGly

4131 GAATAGCCTCTCCACCCAAGCGGCCGAGAACCTGGCTGCAATCCATCTTGTCAATCA  
204 PheLeuArgGlyAlaValTrpAlaAlaProSerGlyAlaHisLeuGlyAspGlyGlyAlaLeu

## BsaBI

## ClaI AvrII

4190 TGGCAAACGATCCTCATCTGCTCTTGATCGATCTTGCAAAAGCCTAGGCCTCCAAA  
04

4249 AAAGCCTCCCTCACTACTCTGGAATAGCTCAGAGGGCGAGGAGGCCCTGGCCCTCTG

4308 CATAAATAAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAACCTGGGGAGTT

Fig. 1 (cont'd III)

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SV40 ori & Promoter

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4367 AGGGGCGGGATGGCCGGAGTTAGGGCGGGACTATGGTGCCTGACTAATTGAGATGCAT NsiI

---

4426 GCCTTGCACTCTGCCTGCTGGGGAGCCTGGGACTTTCCACACCTGGTGCCTGACT SexAI

---

4485 AATTGAGATGCATGCTTGCACTCTGCCTGCTGGGAGCCTGGGACTTTCCACAC NsiI

---

4544 CCTAACTGACACACATTCCACAGCTGGTCTTCCCTCAGGACTTTCTTCAA PvuII Bsu36I

4603 TAAATCAATCTAAAGTATATATGAGTAAACTGGTCTGACAGTTACCAATGCTTAATCA 2871 \*\*\*TrpHisLysIleLe

4662 GTGAGGCACCTATCTAGCGATCTGCTATTCGTTCATCCATAAGTTGCCCTGACTCCCC Eam1105I

2814 uSerAlaGlyIleGluAlaIleGlnArgAsnArgGluAspMetThrAlaGlnSerGlyT

4721 GTCGTGTA GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCCTGCAATGAT 2614 Thr Tyr IleValValIleArgSerProLysGlyAspProGlyLeuAlaAlaIleLe

4780 ACCGGAGACCCACGCTACCGGCTCAGATTTATCAGCAATAAACAGCCAGGCCGAA 4241 GlyArgSerGlyArgGluGlyAlaGlySerLysAspAlaIlePheTrpGlyAlaProLe

4839 GGGCGAGCGCAGAAAGTGGTCTGCAACTTTATCCGCTCATCCAGTCTATTAAATTGT 4839 GGGCGAGCGCAGAAAGTGGTCTGCAACTTTATCCGCTCATCCAGTCTATTAAATTGT

2224 uAlaSerArgLeuLeuProGlyAlaValLysAspAlaGluMetTrpAspIleLeuGlnG FspI PspI406I

4898 TGCGGGGAAGCTAGAGTAAGTAGTTGCCAGTTAATAGTTGCCAACGTTGGCCAT 2024 uArgSerAlaLeuThrLeuLeuGlyThrLeuLeuLysArgLeuThrThrAlaMet

5057 TGCTACAGGCATGTTGTCAGCTCGTCTGCTGGTATGGCTTATTCAAGCTCGGTT 4957 TGCTACAGGCATGTTGTCAGCTCGTCTGCTGGTATGGCTTATTCAAGCTCGGTT

1834 AlaValProMetThrThrAspArgGluAspAsnProIleAlaGluAsnLeuGluProGly 1834 AlaValProMetThrThrAspArgGluAspAsnProIleAlaGluAsnLeuGluProGly

5016 CCCAACGATCAAGGCAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTAGCTCC 5016 CCCAACGATCAAGGCAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTAGCTCC

1634 uTrpArgAspLeuArgThrValHisAspGlyMetAsnHisLeuPheAlaThrLeuGluL 1634 uTrpArgAspLeuArgThrValHisAspGlyMetAsnHisLeuPheAlaThrLeuGluL

PvuI

5075 TTGGTCCCTCGATGTTGTCAGAAGTAAGTTGGCGCAGTGTATCACTCATGGTTAT 1434 ysProGlyGlyIleThrThrLeuLeuLeuAsnAlaAlaThrAsnAspSerMetThrIle

5134 GGCAGCACTGCATAATTCTCTACTGTCATGCCATCGTAAGATGCTTTCTGTGACTG 5134 GGCAGCACTGCATAATTCTCTACTGTCATGCCATCGTAAGATGCTTTCTGTGACTG

1244 AlaAlaSerCysLeuGluArgValThrMetGlyAspThrLeuHisLysGluThrValPro 1244 AlaAlaSerCysLeuGluArgValThrMetGlyAspThrLeuHisLysGluThrValPro

Scal

5193 GTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCCGACCGAGTTGCTCTGC 5193 GTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCCGACCGAGTTGCTCTGC

1044 oSer Tyr GlnValLeuAspAsnGlnSerTyrHisIleArgArgGlyLeuGlnGluGlnG 1044 oSer Tyr GlnValLeuAspAsnGlnSerTyrHisIleArgArgGlyLeuGlnGluGlnG

5252 CGGGCGTCAATACGGGATAATACCGGCCACATACGAGAACTTTAAAAGTGCTCATCAT 5252 CGGGCGTCAATACGGGATAATACCGGCCACATACGAGAACTTTAAAAGTGCTCATCAT

844 IleAlaAspIleArgSerLeuValAlaGlyCysLeuLeuValLysPheThrSerMetMet 844 IleAlaAspIleArgSerLeuValAlaGlyCysLeuLeuValLysPheThrSerMetMet

PspI406I

XbaI

5311 TGGAAAACGTTCTCGGGGGCGAAAACCTCAAGGATCTTACCGCTGTTGAGATCCAGTT 654 PhePheArgGluGluPrArgPheSerGluLeuIleLysGlySerAsnLeuAspLeuGlu

Apal

5370 CGATGTAACCCACTGTCACCAACTGATCTTCAGCATCTTACTTTACCCAGCGTT 5370 CGATGTAACCCACTGTCACCAACTGATCTTCAGCATCTTACTTTACCCAGCGTT

4544 uIleTyrGlyValArgAlaGlyLeuGlnAspGluAlaAspLysValLysValLeuThrG 4544 uIleTyrGlyValArgAlaGlyLeuGlnAspGluAlaAspLysValLysValLeuThrG

5429 TCTGGGTGAGAAAAACAGGAAGGAAAATGCCGCAAAAAGGGATAAGGGCAGACG 5429 TCTGGGTGAGAAAAACAGGAAGGAAAATGCCGCAAAAAGGGATAAGGGCAGACG

2544 uProHisAlaPheValProLeuCysPheAlaAlaPhePheProIleLeuAlaValArg 2544 uProHisAlaPheValProLeuCysPheAlaAlaPhePheProIleLeuAlaValArg

SspI

5488 GAAATGTTGAATACTCATACTCTTCTTTCAATATTATTGAAGCATTATCAGGGTT 6488 GAAATGTTGAATACTCATACTCTTCTTTCAATATTATTGAAGCATTATCAGGGTT

6488 PheHisGlnIleSerMet

BspHI

5547 ATTGTCTCATGAGCGGATACATATTGAATGTATTAGAAAAATAACAAATAGGGTT 5547 ATTGTCTCATGAGCGGATACATATTGAATGTATTAGAAAAATAACAAATAGGGTT

5606 CGCGCACATTCCCCGAAAAGTCCCACCTGACGCCCTGTAGCGGGCGATTAGCGCGC 5606 CGCGCACATTCCCCGAAAAGTCCCACCTGACGCCCTGTAGCGGGCGATTAGCGCGC

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Stem loop A

5665 GGCGGGTGTGGTGGTTACGGCGAGCGTACACTGCCAGCGCCCTAGCGCCCG 5665 GGCGGGTGTGGTGGTTACGGCGAGCGTACACTGCCAGCGCCCTAGCGCCCG

Fig. (cont'd IV)

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5724 CTCCTTCGTTTCTTCCCTCTTCTGCCACGTTCCCCGGTTCCCCGTCAAGCT

5783 CTAAATCGGGGGCTCCCTTAGGGTCCGATTAGTGCCTTACGGCACCTCGACCCCAA

5842 AAAACTTGATTAGGGTATGGTCACGTAGTGGCCATGCCCTGATAGACGGTTTTC

5901 GCCCTTGACGTTGGAGTCCACGTTCTTAATAGTGGACTCTTGTCCAAACTGGAAACA

5960 AGACTCAACCTATCTGGTCTATTCTTGATTATAAGGGATTTGCCGATTCGGC

6019 CTATTGGTTAAAAAATGAGCTGATTAAACAAAATTAACCGGAATTAAACAAAATAT

6078 TAACGCTTACAATTAC

Fig. 1 (cont'd V)

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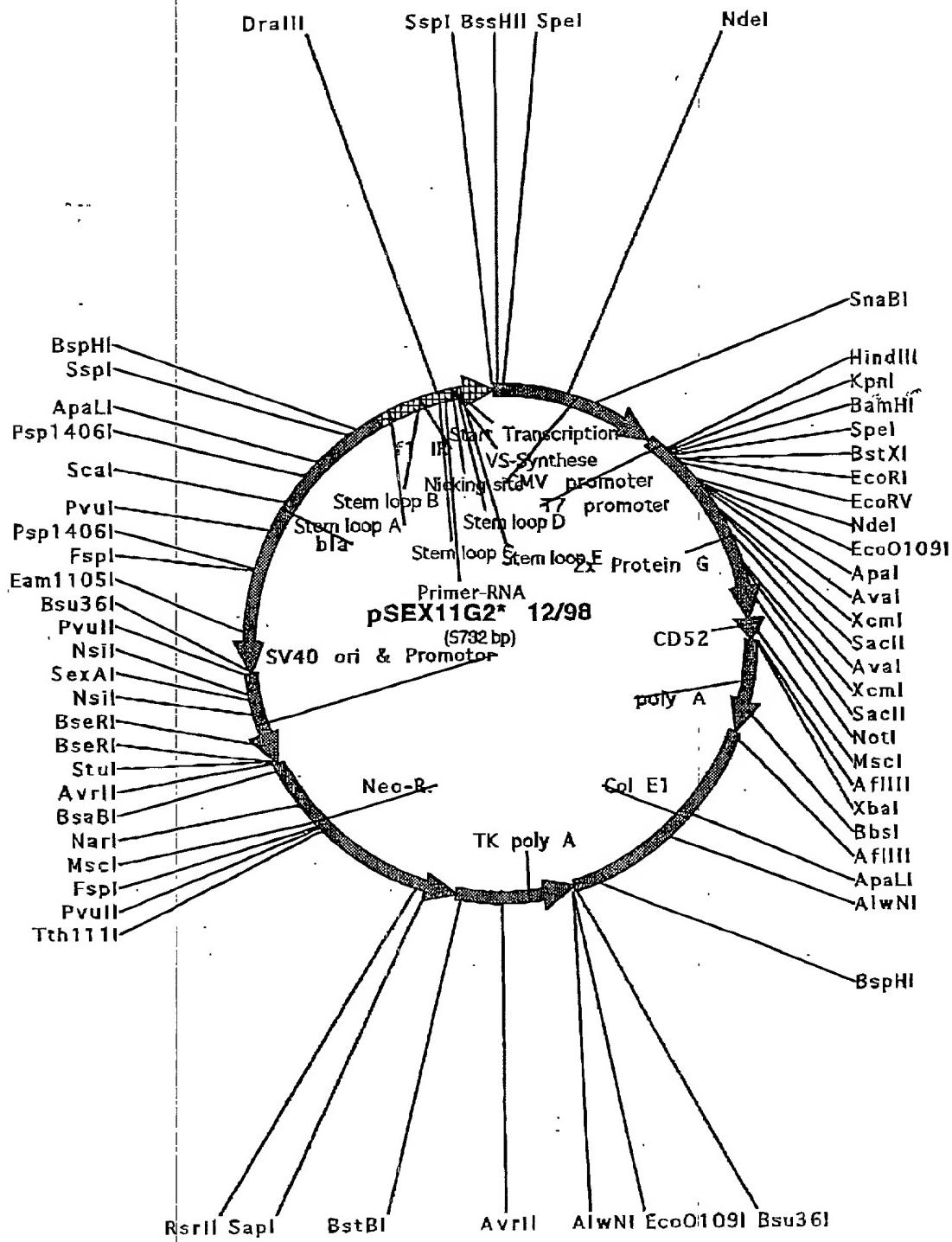


Fig. 2

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Fig. 2 (cont'd I)

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1081 CGCCGTGACCACCTACAAGCTAGT GATCAACGGCAAGACCC TGAAGGGCAGAC  
115 ▶ aAl aVal Thr Thr Tyr Lys Leu Val Ile Asn Gly Lys Thr Leu Lys Gly Glu Th

XcmISacII

1135 CACCA CCGAGGCCGTGGACGCCACCGCGGAGAAGGT GTCAAACAAATACGC  
133 ▶ r Thr Thr Gl uAl aVal A spAl aAl aThr Al aGl uLys Val Phe Lys Gl n Tyr Al  
1189 TAAT GACAACAGGGGTGACGGGAGGTGGACTTACGACGAGCCACCAAGACCTT  
151 ▶ aAsn Asp Asn Gly yVal A spGly Gl uTr pThr Tyr Asp Asp Al aThr Lys Thr Ph

NotI

1243 CACCGT GACCGAGGCCGGCGAGAACAAA ACTCATCT CAGAAGAGGA TCTGAA  
169 ▶ eThr Val Thr Gl uAl aAl aAl aGl uGl n Lys Leu Ile Ser Gl uGl uAsp Leu As

1297 TGGGGCCGTCGACGGACAAAGACACCCAGCCAAACCCAGCAGCCCCCTCAGCATC  
187 ▶ nGly Al aVal Asp Gl yGl nAsn Asp Thr Ser Gl n Thr Ser Ser Pro Ser Al aSe

CD52MscI

1351 CAGCAACATAAGCGGAGGCATTTCCTTTCTTCGTGGCAATGCCATAATCCA  
205 ▶ rSer Asn Ile Ser Gl yGl Ile Phe Leu Phe Phe Val Al aAsn Al al Ile Hi

AfIII/XbaI

1405 CCTCTTCTGCTTCA GTT GAGGT GACAC GTCTAGAGCTATTCTATAGT GTCACCT  
223 ▶ sLeu Phe Cys Phe Ser \*\*\* ←

1459 AAATGCTAGAGCTCGT GATCAGCCTCGACTGTGCCCTCTAGTTGCCAGCCATC ←

1513 TGTGTTTGCCCCCTCCCCGTGCCCTCTGACCCCTGGAAAGGTGCCACTCCAC ←

poly A

1567 TGTCCTTCTCTAAATAAAATGAGGAAATTGCATCGATTGCTGAGTAGGTGTC ←

BbsI

1621 TTCTATTCTGGGGGTGGGGTGGGGCAGGA CAGCAAGGGGAGGATTGGGAAGA

1675 CAATAGCAGGCATGCTGGGGATCCGGTGGGCTCTATGGCTCTGAGGCCGGAAAG

1729 AACAGTGGCGTAATACGGTTATCCACAGAATCAGGGATAACGCAGGAAAGA

AfIII

1783 ACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAGGCCGGTTGC

1837 TGGCGTTTCCATAGGCCTCCCCCCCCTGACGGAGCATCACAAAAATCGACGCT

1891 CAAGTCAGAGGTGGC GAAACCCGACAGGACTATAAGATA ACCAGCGTTCCC

1945 CTGGAA GCTCCCTCGCGCTCTCTGTTCCGACCC TCCGCTTACCGGATACC

1999 TGTCCGCTTTCTCCCTCGGGAAAGCGTGGCGTTCTCATAGCTCACGCTGTA

ApalI

2053 GGTATCTCAGTTGGTGTAGGTGTTGCTCCAGCTGGCTGTGCA CGAAC

Col E1

2107 CCCCCGGTTAGCCCCGACCGCTGCCCTTATCGGTAACTATCGCTT GAGTCCA

AIwNI

2161 ACCCGGTAAGACACGGACTTATGCCACTGGCAGCAGCCACTGGTAACAGGATTA

2215 GCAGAGCGAGGTATGAGGC GGCTACAGAGTTCTGAAGTGGTGGCTAACT

2269 ACGGCTACACTAGAAGGA CAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTA

2323 CCTTCGGAAAAAGAGTTGGTAGCTCTGATCCGGCAAACAAACCACCGCTGGTA

Fig. 2 (cont'd II)

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Fig. 2 (cont'd III)

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BseRI  
 3889 AGCTCCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGAGGCCCTCGGCCT

---

3943 CTGCATAAATAAAAAAAATTAGTCAGCCATGGGCCGGAGAATGGCGGAACCTGG

---

3997 GCGGAGTTAGGGCGGGATGGCGGAGTTAGGGCGGGACTATGGTGCTGACT

---

Nsil  
 4051 ATTGAGATGCATGCTTGCATACTTCTGCCTGCTGGGAGCTGGGACTTT

---

SexAI Nsil  
 4105 CACACCTGGTTGCTGACTAATTGAGATGCATGCTTGCATACTTCTGCCTGCTG

---

PvuII  
 4159 GGGAGCCTGGGACTTCCACACCTAACGTACACACATTCCACAGCTGGTCT

---

Bsu36I  
 4213 TTCCGCCTCAGGACTCTTCTTTCAATAATCAATCTAAAGTATATGAGT

4267 AAACCTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGA

2871 TrpHisLysIleLeuSerAlaGlyIleGluAlaI

Eam1105I  
 4321 TCTGTCTATTCGTTCATCCATAGTTGCCGACTCCCCGCGTAGATAACTA

274 ArgAsnArgGlyIleAspMetThrAlaGlyIleSerGlyThrThrTyrIleValVa

4375 CGATACGGGAGGGCTTACCATCTGGCCCCACTGCTGCAATGATACCGCGAGACC

256 IleArgSerProLysGlyAspProGlyIleLeuAlaAlaAlaIleGlyArgSerGly

4429 CACGCTCACGGCTCCAGATTATCAGCAATAAACCCAGCCAGCCGGAGGGCCG

238 ArgGlyIleGlyAlaGlySerLysAspAlaIlePheTrpGlyIleAlaProLeuAlaSer

4483 AGCGCAGAAGTGGCTCTGCAACTTATCCGCTCCATCCAGTCTATTAAATTGTT

220 ArgLeuLeuProGlyAlaValLysAspAlaGlyMetTrpAspIleLeuGly

FspI Psp1406I  
 4537 GCCGGGAAGCTAGAGTAAGTCTGCCAGTTAATAGTTGGCAACCGTTGTTG

202 ArgSerAlaLeuThrLeuLeuGlyIleThrLeuLeuLysArgLeuThrThrAla

4591 CCATTGCTACAGGCATCGGGTGTACGCTCGTCGTTGGTATGGCTTCATTCA

184 MetAlaValProMetThrThrAspArgGlyIleAspAsnProIleAlaGlyAsnLe

4645 GCTCCGGTTCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAA

166 GlyIleProGlyIleTrpArgAspLeuArgThrValHisAspGlyMetAsnHisLeuPh

PvuI  
 4699 AAGCGGTTAGCTCTTCCGCTCCGATCGTGTCAAGAAGTAAGTTGGCCGCAG

148 AlaIleThrLeuGlyIleLysProGlyIleThrThrLeuLeuAsnAlaAlaTh

4753 TGTTATCACTCATGGTTATGGCAGCACTGCTATAATTCTTACTGTCATGCCAT

130 AsnAspSerMetThrIleAlaAlaSerCysLeuGlyArgValThrMetGlyAs

bla Scal  
 4807 CGCTAAGATGCTTTCTGTGACTGGTGAAGTCAACCAAGTCATTCTGAGAAT

112 pThrLeuHisLysGlyIleThrValIleProSerTyrGlyIleValLeuAspAsnGlyIleSerTy

4861 AGTGATGGCGCACCGAGTTGCTCTGGCCGGCGTCATAACGGGATAATACCG

94 HisIleArgArgGlyIleLeuGlyIleGlyIleAspIleArgSerLeuValAla

Psp1406I  
 4915 CCCACATAGCAGAACCTTAAAGTCTCATCTGGAAACGTTCTGGGGC

78 ArgGlyCysLeuLeuValLysPheThrSerMetMetProPheArgGlyIleGlyIleAspAla

4969 GAAACTCTCAAGATCTTACCGCTGTTGAGATCCAGTCGATGTAACCCACTC

58 ArgPheSerGlyIleLeuIleLysGlyIleSerAsnLeuAspLeuGlyIleTyrGlyValAla

Apal  
 5023 GTGCACCCAACGATCTTCAGCATCTTCTACCAAGCGTTCTGGGTGAG

40 ArgAlaGlyIleLeuGlyIleAspGlyIleAspLysValIleValLeuThrGlyIleProHisAla

5077 CAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGATAAGGGCGACACGGAAAT

22 aPheValProLeuCysPheAlaAlaPhePheProIleLeuAlaValArgPheHis

SspI  
 5131 GTTGAATACTCATACTCTTCTTTCAATATTATTGAAGCATTATCAGGGTT

44 GlyIleSerMet

BspHI  
 5185 ATTGTCTCATGAGCGGATACTATTGAATGTATTAGAAAAATAACAAATAG

Fig. 2 (cont'd IV)

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5239 GGGTCCGCCACATTTCCCCAAAAGGCCACCTGACGCCCTGTAGCGGCG

Stem loop A

5293 CATTAAGCGCGGGTGTGGTGGTACGCCAGCGTGAACGCTACCTGCCA

5347 GCGCCCTAGGCCCGCTCTTCGCTTCTCCCTCTGCCACGTTG

f1 IR Stem loop B

5401 CCGGCTTCCCCGTCAAGCTAAATCGGGGCTCCCTTAGGGTCCGATTAA

DraIII

5455 GTGCTTACGGCACCTGACCCAAAAAACTTGATTAGGGTATGGTACGTA

5509 GTGGGCCATGCCCTGATAGACGGTTTCGCCCTTGACGTTGGAGTCACGT

Start Transcription

Stem loop C

Primer-RNA

VS-Syntheis

5563 TCTTAATAAGTGGACTCTGTTCAAACCTGGAAACAACCTAACCTATCTGG

Nicking site Stem loop D Stem loop E

5617 TCTATTCTTTGATTATAAGGGATTTGCCGATTTGCCCTATTGGTAAAAAA

SspI

5671 ATGAGCTGATTTAACAAAAATTAAACCGCAATTAAACAAATATTAAACGCTTA

5725 CAATTTAC

Fig. 2 (cont'd V)

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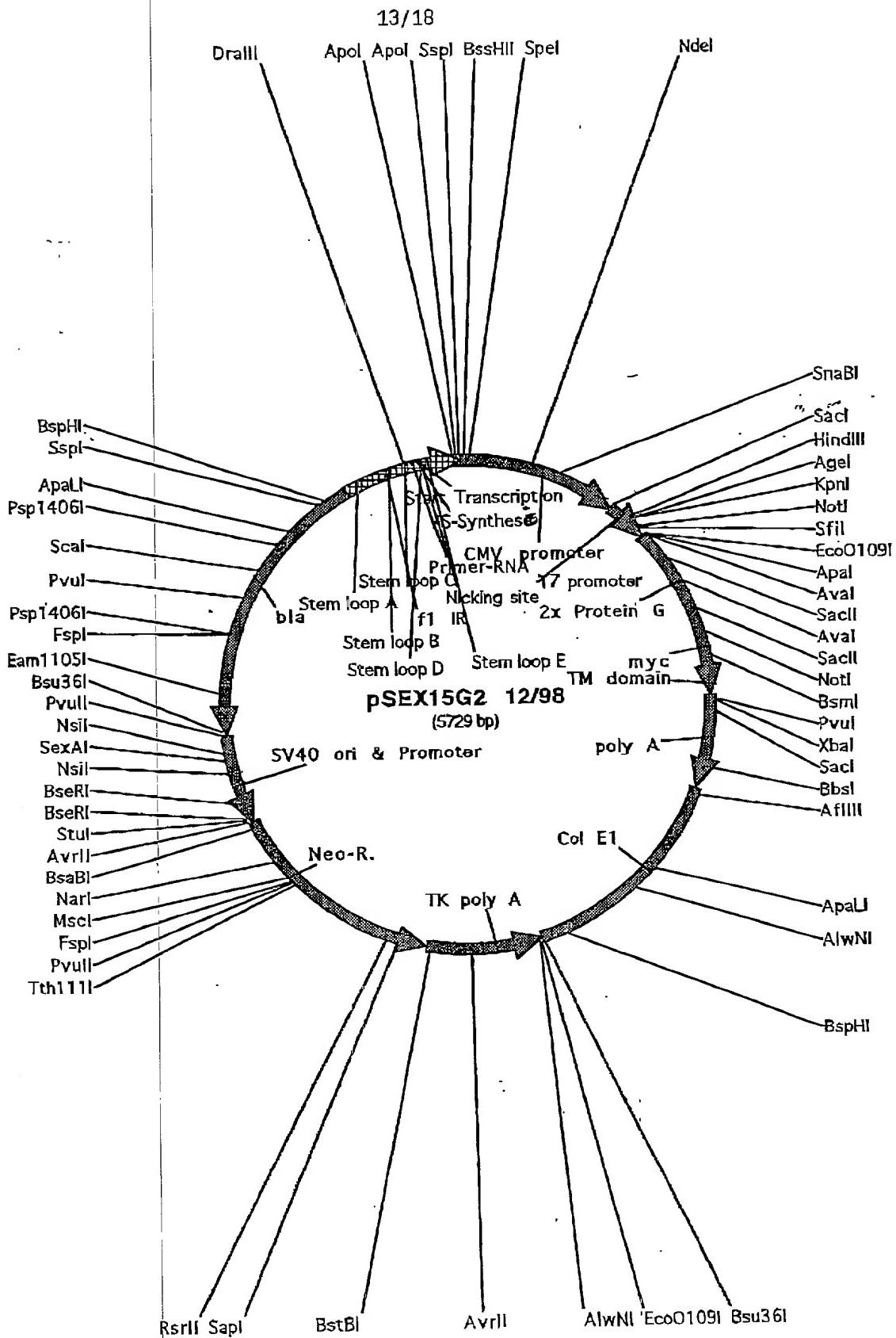


Fig. 3

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Fig. 3 (cont'd 1)

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Not  
1121 TCGACGGCAGTGGACTTACGACGCCACCAAGACCTCACCGTGACCGAGGCG  
147>AlaAspGlyGluTrpThrTyrAspAspAlaThrLysThrPheThrValThrGluAla

myc  
1177 GCCGCAGAACAAAAACTCATCTAGAAGAGGATCTGAATGGGCCGTGACGAACA  
166>AlaAlaGluGlnLysLeuIleSerGluGluAspLeuAsnGlyAlaValAspGluGlu

BsmI  
1233 AAAACTCATCTAGAAGAGGATCTGAATGCTGTGGGCCAGGACACGAGGAGTC  
184>nLysLeuIleSerGluGluAspLeuAsnAlaValGlyGluAspThrGluValI

1289 TCGTGGTCCCACACTCTTGCCTTAAGGTGGTGGTGTCTAGCCATCTGGCC  
203>IleValValProHisSerLeuProPheLysValValValIleSerAlaIleLeuAla

TM domain  
1345 CTGGTGGTGTCAACATCATCTCCCTTATCATCCTCATGCTTGGCAGAAGAA  
222>LeuValValLeuThrIleIleSerLeuIleIleLeuIleMetLeuTrpGlnLysLy

PvuI XbaI  
1401 GCCACGTTCTCGCCGATCGAGAATCCATCTAGAGCTATTCTATAGTGTACCTA  
240>sProArgSerSerAlaAspArgGluSerIle\*\*\* ←

SacI  
1457 AATGCTAGAGCTCGCTGATCAGCTCGACTGTGCCTTAGTTGCCAGCCATCTGT ← poly A

1513 TGTTTGCCTCCCCGTGCCTTCCTTGACCCCTGGAAAGGTGCCACTCCACTGTCC

1569 TTTCTAATAAAATGAGGAATTGCATCGATTGCTGAGTAGGTGTCAATTATT

BbsI  
1625 CTGGGGGGTGGGTGGGCAGGACAGCAAGGGGAGGATGGGAAGACAATAGCAG

1681 GCATGCTGGGATGCGGTGGCTATGGCTCTGAGGCCAAAGAACCACTGGCG

ApaI III  
1737 GTAATACGTTATCCACAGAACATCAGGGATAACCCAGGAAAGAACATGTGAGCAA  
1793 AGGCCAGAAAAGGCCAGGAACCGTAAAAGGCCGCGTGGCTGGCTTTCCATA

1849 GGCTCCGCCCCCTGACGAGCATCACAAATGACGCTCAAGTCAGAGGTGGCGA

1905 AACCCGACAGGACTATAAGATACCAAGGGCTTCCCCCTGGAGCTCCCTGTGCG

1961 CTCTCCGTCCGACCCCTGCCCTAACCGATACTGTCCGCCCTTCTCCCTCGG

2017 GAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTCGGTGTAGGTC

ApaII Col E1  
2073 GTTCGCTCCAAGCTGGCTGTGACGAACCCCCGTTCAAGCCGACCGCTGCGC

2129 CTTATCCGTAACATCGTCTGACTGCAACCCGTAAGACACGACTTATGCCAC

A1wNI  
2185 TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGAGGCGGTGCTACA

2241 GAGTTCTTGAAGTGGTGGCTAACTACCGCTACACTAAGAAGGACAGTATTGCTAT

2297 CTGCGCTCTGCTGAAGCCAGTTACCTTGGAAAAAGAGTTGGTAGCTCTGATCCG

2353 GCAAACAAACCACCGCTGGTAGGGTGGTTTTGTTGCAAGGAGCAGATTACG

2409 CGCAGAAAAAAAGGATCTAAGAAGATCCTTGTATCTTCTACGGGTCTGACGC

Fig. 3 (cont'd II)

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BspHI

2465 TCA GTGG ACG AAA ACT CAC GTT AAG GG AT TT GGT CAT GAG ATT AT CAAA AGGA  
 2521 TCT T CAC CT AGAT CTT TAA ATT AAA AT GAAG TTT AA AT CA AT CT AA AGT ATA

EcoO109I

2577 TAT GAG TAAC CTG AGG CT AT GG CAG GG CT GCC CCC GAC GT GG CT GGG AG CCC

Bsu36I AlwNI

2633 TGG GCCT TCAC CC GA AC TT GGG GGT GGG GAA AGG AAG AAG CCG GGG CGT

2689 ATT GGC CCA AT GGG TCT CGG TGG GT AT CGA CAG AGT GCC AG CCT GGG ACC GA

TK poly A

2745 ACC CCG CTT AT GAAC AA AC GAC CCA AC CCT GT GC GT TT ATT CT GT CTT TT AT

2801 TGC CGT CA TAG CGC GGG TT CCT CGG TATT GT CT CCT CGT GT TT CAG TT AGCC

AvrII

2857 TCCCCCTAGGGTGGCGAAGAACCTCAGCATGAGATCCCCCGCTGGAGGATCATC

2913 CAG CCG CGT CCCC GAA AAC GAT TCC GAAG CCA AC CCT TCA TAG AAG GCG CGG T

BstBI

2969 GGA AT CGA AA AT CT CGT AT GG CAG GT GGG CGT CGT TGG TCA IT TCG AACC  
 3025 CCAG AGT CCC CGT CAG AAG AACT CGT CAA GAG GCG AT AGA AGG CG AT CGT CG  
 2634 \*\*\* Phe Gl uAsp Leu Arg Tyr Phe Al l le Arg Gl nSe  
 3081 AAT CGG GAG CGG CG AT ACC GT AA AG CAC CG AGG AAG CG GT CAG CCC ATT CG CG CC A  
 2481 rAsp Pro Al a Al l le Gly Tyr Leu Val Leu Phe Arg Asp Al a Tr p Gl u Gly Gl y L

SapI

3137 AGCT CTT CAG CAAT AT CAC GGG TAG CCA AC GCT AT GTC CTG AT AGC GGT CCC CAC

2291 eu Gl u Gl u Al a Al l e Asp Arg Thr Al a Leu Al l l e Asp Gl n Tyr Arg Asp Al a Val

3193 ACC CAG CGC CC ACAG TCG AT GAAT CCAG AAA AGC GGC CAT IT TCC ACC AT GAT AT

2114 Gl y Leu Arg Gl y Cys Asp l l e Phe Gl y Ser Phe Arg Gl y Asn Gl u Val Met l l e As

3249 TCG GCA AG CAG GC AT CGC CAT GGG T CAG CAG GAG AT CCT CG CC GT CG GG CAT GCT C

1924 nPro Leu Cys Al a Asp Gly His Thr Val Val Leu Asp Gl u Gl y Asp Pro Met Ser A

3305 GCCT TGAG CCT CG CG A AC AGT TCG CTG CG GAG CCCC CTG AT GCT CCT GAT CTC

1734 l a Lys Leu Arg Al a Phe Leu Gl u Al a Pro Al a Leu Gl y Gl n Hi s Gl u Gl n Asp Asp

3361 CTG AT CGA CA AG ACG CG CT TCC AT CG AGT AC GT GCT CG CT CG AT GGG AT GT TT CG

1554 Gl n Asp Val Leu Gl y Al a Gl u Met Arg Thr Arg Al a Arg Gl u l e Arg His Lys Al

3417 CTT GGT GGT CGA AT GGG CAG GT AG CCG GAT CAAG CGT AT GCA GCG CG CC GAT T GCA

1364 a Gl n Hi s Asp Phe Pro Cys Thr Al a Pro Asp Leu Thr Hi s Leu Arg g Arg Me l Al a A

3473 TCAG CC AT GAT GGA TACT TCT CGG CAG GAG CA AG GT GAG AT GA CAG GAG AT CCT G

1174 sp Al a Met l l e Ser Val Lys Gl u Al a Pro Al a Leu Hi s Ser Ser Leu Leu Asp Gl n

Tth111

3529 CCCCCGG CACT TCG CCC AAT AGC AG CC AG TCC CT TCC CG CT TCA GT GAC AAC GT CG A

99 Gl y Pro Val Gl u Gl y Leu Leu Leu Tr p Asp Arg Gl y Al a Gl u Thr Val Val Asp Le

Neo-R.

PvuII/FspI

3585 GCAC AG CT CG CG CA AG GA AC GCG CC CGT CG TGG C CAG CC AC GAT AG CCG GCG CT GCG CT CG

801 u Val Al a Al a Cys Pro Val Gl y Thr Thr Al a Leu Tr p Ser Leu Arg Al a Al a Gl u A

NarI

3641 TCT TG CAG TT CATT CGG CAC CGG AC AG GT CGG TCT TGA CAAA AGA AC CG GGC G

614 sp Gl u Leu Gl u Asn Leu Al a Gl y Ser Leu Asp Thr Lys Val Phe Leu Val Pro Arg

3697 CCC CT CG CG TA CAG CG CG GA AC AG CG CG C GAT CG AG GC AG CG GAT T GT CT GT GT G

434 Gl y Gl n Al a Ser Leu Arg Phe Val Al a Al a Asp Ser Oys Gl y l l e Thr Gl n Gl n Al

3753 CCC AGT CA TAG CG GA AT AGC CT CCT CAC CCA AG CG CG CC GG AGA AC CT CG GT GCA AT

244 a Tr p Asp Tyr Gl y Phe Leu Arg Gl u Val Tr p Al a Al a Pro Ser Gl y Al a Hi s Leu G

BsaBI

3809 CCAT CTT GTT CA AT CAT CG A A A C G AT CCT C AT CCT GT CT T GAT CG AT CCT T G

541 y Asp Gl n Gl u l l e Met

StuI

AvrII

3865 AAA AGC CTAGG CCT CCA AAA AGC CT CT ACT ACT TCT GGA AT AGC T CAG AGG G C C

Fig. 3 (cont'd III)

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3921	BserI GAGGAGGGGGCTCGGCCTGCAATAAATTTAGTCAGCCATGGGGCGG
3977	SV40 ori & Promoter AGAATGGCGGAACCTGGCGGAGTTAGGGCGGATGGCGGAGTTAGGGCGGGA
4033	NsiI CTATGGTTGCTGACTAATTGAGATGCATGCTTGCATACTTCTGCCTGCTGGGAG
4089	SexAI CCTGGGGACTTCCACACCTGGTGTGACTAATTGAGATGCATGCTTGCATACT
4145	PvuII TCTGCCTGCTGGGGAGCCTGGGACTTCCACACCTAAC TGACACACATTCCACA
4201	Bsu36I GCTGGTTCTTCGGCTCAGGACTCTTCAATAATCAATCTAAAGTATA
4257	TATGAGTAAACTTGGTGTGACAGTTACCAATGCTTAATCAGTGAGGACCTATCTC 2871***TrpHisLysIleLeuSerAlaGlyIleGlu**
4313	Eam115I AGCGATCTGCTATTCGTTCATCCATAGTGCCTGACTCCCCGTCGTAGATAA
2761	AlaIleGlnArgAsnArgGluAspMetThrAlaGlnSerGlyThrThrTyrIleVal
4369	CTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCATGATACCGCAGAC
2574	IValIleArgSerProLysGlyAspProGlyIleLeuAlaAlaIleIleGlyArgSerG
4425	CCACGCTCACCGGCTCAGATTATCAGAACAAAACCAGGCCAGCCGGAAAGGGCCGA
2381	IlyArgGlyIlaGlySerLysAspAlaIlePheTrpGlyAlaProLeuAlaSer
4481	GCGCAGAAGTGGTCTGCAACTTATCCGCTCCATCCAGCTATTAAATTGTTGCC
2201	ArgLeuLeuProGlyAlaValLysAspAlaGluMetTrpAspIleLeuGlyIleGlu
	FspI Psp1406I
4537	GGGAAGCTAGAGTAAGTAGTTCGGCCAGTTAATAGTTGGCCAACGTTGTCATT
2011	IgSerAlaLeuThrLeuLeuGlyIleGlyThrLeuLeuLysArgGluThrThrAlaMetA
4593	GCTACAGGCATCGTGTGTCACGCTCGTCTGGTATGGCTTCATTGCTCCGG
1821	IaValProMetThrThrAspArgGlyIleAspAsnProIleAlaGlyIleAsnLeuGlyIlePro
4649	TTCCCAACGATCAAGGGCAGTTACATGATCCCCATGTTGCAAAGAAAGCGGTTA
1641	GlyIleTrpArgAspLeuArgThrValHisAspGlyMetAsnHisLeuPheAlaThrLe
	PvuI Psp1406I
4705	GCTCTTCGGCTCTCGATCGTGTGAGAACGTAAGTTGGCCGAGTGTATCACTC
1451	uGluLysProGlyGlyIleThrThrLeuLeuLeuAsnAlaAlaThrAsnAspSerM
	bla
4761	ATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAAGATGCTT
1261	IleThrIleAlaAlaSerCysLeuGlyIleArgValThrMetGlyIleAspThrLeuHiLys
	Scal
4817	TTCTGTGACTGGTGACTCAACCAAGTCATTCTGAGAATAGTGTATGGCCGAC
1081	GlyIleThrValProSerTyrGlyIleValLeuAspAsnGlnSerTyrHisIleArgGlyIle
4873	CGAGTTGCTCTGGCCGGCGTCAATACGGGATAATACCGGCCACATAGCAGAACT
891	IleGlyLeuGlyIleGlyAlaAspIleArgSerLeuValAlaGlyIleCysLeuLeuValL
	Psp1406I
4929	TTAAAAGTGTCTCATTTGGAAAAACGTTCTGGGGCGAAAACCTCTCAAGGATCTT
701	IysPheThrSerMetMetProPheArgGlyIleGluIleProArgPheSerGlyIleLeuIleLys
	ApalI
4985	ACCGCTGTGAGATCCAGTTGATGTAACCCACTCGTCACCCACTGATCTTCAG
521	GlySerAsnLeuAspLeuGlyIleTyrGlyIleValArgAlaGlyIleLeuGlyIleAspGlyIle
5041	CATCTTTACTTCAACAGCGTTCTGGTGAGCAGGAAACAGGAAGGCAAAATGCC
331	IaAspLysValLysValLeuThrGlyIleProGlyIleAlaPheValProLeuCysPheAlaA
5097	GCAAAAGGGATAAGGGCAGACGGAAATGTTGAATACTCATACTCTCTCTT
141	IaPhePheProIleLeuAlaValArgPheHisGlyIleSerMet
	SspI
5153	TCAATTATTGAAAGCATTATCAGGGTTATGTCATGAGCGGATACATATTG
5209	AATGTATTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTCCCGAAAGTG
5265	CCACCTGACGCCCTGTAGCGCGCATTAAAGCGGGCGGGTGTGGTGTAAAGCG

Fig. 3 (cont'd IV)

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5321 Stem loop A  
CAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGTCCCTTCGCTTCTTCC

5377 CTTCCTTCTGCCACGTTGCCGGCTTCCCCGTCAAGCTAAATCGGGGGCTC

5433 f1 IR Stem loop B  
CCTTAGGGTTCCGATTAGTGCCTTACGGCACCTGACCCAAAAAATTGATTA

5489 Drall Stem loop C Primer-RNA  
GGGTGATGGTTACGTAGTGGCCATGCCCTGATAGACGGTTTCGCCCTTGA

5545 Start Transcription VS-Synthes Nicking site Stem loop D Stem loop E  
CGTTGGAGTCCACGTTCTTAATAGTGGACTCTGGTCCAAACTGGAACAAACACTC

5601 AACCTATCTGGCTATTCTTGTATTAAGGGATTTGCCGATTCGGCCTA

5657 Apol Apol SspI  
TTGGTTAAAAATGAGCTGATTAACAAAATTAAACGCGAATTAAACAAAATAT

5713 TAACGCTTACAATTAC

Fig. 3 (cont'd V)